Detection (SKY)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Avidin-Cy5 (1.8 mg/ml)
Jackson Immuno Research Lab, Cat. 003-170-083
Bovine Serum Albumin (BSA)
Roche Diagnostics, Cat. 100-350
DAPI
Sigma, Cat. 18860
Ethyl alcohol, anhydrous
Formamide
Fluka BioChemika, Cat. 47671
HCl, 1 N
Mouse anti-digoxigenin (0.1 mg/ml)
Sigma, Cat. D 8156
Sheep anti-mouse Cy5.5 (1.0mg/ml)
Rockland Immunochemicals, Cat. 610-113-121
SSC, 20X
Tween 20
Sigma, Cat. P-1379
dH_2O
Antifade (1,4-pheylene-diamine)
Sigma, Cat. P1519, 100 g

Preparation

50% FA/2X SSC
20X SSC 20 ml
dH_2O 80 ml
Formamide 100 ml
Adjust pH to 7.25 using 1 N HCl
Pre-warm to 45°C

1X SSC
20X SSC 25 ml
dH_2O 475 ml
Pre-warm to 45°C

4X SSC/0.1%Tween 20
20X SSC 200 ml
dH_2O 799 ml
Tween 20 1 ml
Pre-warm to 45°C

Blocking Solution (3% BSA/4X SSC/0.1%Tween 20)
BSA 0.3 g
4X SSC/0.1%Tween 20 10 ml
Vortex until dissolved
Pre-warm to 37°C

Antibody Solution (1% BSA/4X SSC/0.1%Tween 20)
0.1 g BSA
10 ml 4X SSC/0.1%Tween 20
Pre-warm to 37°C
(or use 4X SSC/ 0.1% Tween 20; see note 4)

DAPI stock solution (f.c.= 0.2 mg/ml)
DAPI 2 mg
dH_2O 10 ml
Aliquot and store at -80ºC

DAPI staining solution (f.c.= 80 ng/ml)
DAPI (stock solution) 40 µl
2X SSC 100 ml
Store at 4°C in a light-tight coplin jar.

Procedure

1. Carefully remove rubber cement surrounding coverslips with forceps. Pre-soak slide in formamide/2X SSC if rubber cement is difficult to remove.

2. Wash slides in 50% formamide/2X SSC for 3 x 5 min each, shaking, preferably in 45°C water-bath.

3. Wash slides in 1X SSC for 3 x 5 min, shaking.

4. Dip slides in 4X SSC/0.1% Tween 20; do not let them dry.

5. Add 120 µl of Blocking Solution (3% BSA/4X SSC/0.1%Tween20) to a 24 mm x 60 mm coverslip and incubate in a moist hybridization chamber at 37°C for 30 min.

6. Wash slides in 4X SSC/0.1% Tween 20 to wash off blocking solution, 5 min, shaking.

7. Spin all fluorescent dyes for 1 min at 13,000 rpm.

8. Combine the two antibodies, mouse anti-Dig and Avidin-Cy5, into the same eppendorf tube, and apply 120 µl of antibody solution to a 24 mm x 60 mm coverslip. Each antibody should be diluted 1:200 in 1% BSA/4X SSC/0.1% Tween 20 or 4X SSC/0.1% Tween 20 (see note 4). Invert the slide onto the solution. Incubate the slides in a moist hybridization chamber at 37°C for 45-60 min.

9. Wash slides in 4X SSC/0.1% Tween 20, 3 x 5 min, shaking.

10. Add 120 µl of the antibody (sheep anti-mouse Cy5.5, diluted 1:200 in 1% BSA/4X SSC/0.1% Tween 20 or 4X SSC/0.1% Tween 20). Incubate slides in a moist hybridization chamber at 37°C for 45-60 min.

11. Wash slides in 4X SSC/0.1% Tween 20, 3 x 5 min, shaking.

12. Stain slides for 5 min in the DAPI staining solution in a light-protected coplin jar at RT.

13. Wash slides with 2X SSC 3-5 min.

14. Dehydrate slides in ethanol series of 70%, 90%, and 100% for 3 min each, air-dry slides.

15. Apply 35 µl of antifade solution, cover each slide with a 24 mm x 60 mm coverslip, and store in a light-protected container at 4˚C until slide is imaged.

Notes

1. Exposure of slides to ambient light should be minimized during all procedures.

2. Carefully remove coverslips during all procedures to minimize scratches.

3. Do not let the slide dry out between washing steps.

4. BSA may contribute to non-specific background.